UNIVERSITI PUTRA MALAYSIA

INDUCED RESISTANCE USING NONPATHOGENIC FUSARIUM OXYSPORUM FOR BIOLOGICAL CONTROL OF BANANA FUSARIUM WILT

TARIG ELSAYED ALI ELSHARIF

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By

TARIG ELSAYED ALI ELSHARIF

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirement for the Degree of Doctor of Philosophy

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DEDICATION

I dedicate this humble effort, the fruit of my thoughts and study to my affectionate Mother, Father, wife Nagwa and children who inspired me to higher ideals of life.

Abstract of thesis submitted to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Doctor of Philosophy

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Chairperson : **Professor Dr. Sariah Meon**

Faculty : **Agriculture**

Banana (*Musa* spp. Linn.) is the second most important fruit crops in Malaysia. It is easily attacked by the fungus *Fusarium oxysporum* Schlect. f.sp. *cubense* (E.F.Smith) Snyder and Hansen (FOC), causing terminal wilt in the field. Chemical and cultural methods were not effective in controlling the disease. Therefore, alternative control measures have to be developed. In recent years, nonpathogenic Fusaria was being considered for plant disease control and could be the most promising approach for biological control of *Fusarium* wilt in banana through induced systemic resistance.

Isolates of nonpathogenic *F. oxysporum* (FO: FO1, FO2, FO3, FO4, FO5 and FO6) were isolated from healthy roots and rhizospheres of bananas vars. *Berangan* and *Rastali*, and were identified to the species level based on cultural and morphological characteristics. Random amplified polymorphic (RAPD–PCR) analysis was able to

establish variability within *F. oxysporum* isolates and between saprophytic and pathogenic forms (FOC race 1 and race 4), using OPC 11 and OPC 14 primers. All 6 isolates of FO were antagonistic to both pathogenic race 1 and race 4 of FOC with values of the % of inhibition of radial growth (PIRG) exceeding 50% in a series of dual culture test. FO4 was found to be the most antagonistic against FOC race 4 with PIRG of 65%.

Infectivity studies on six-weeks-old tissue cultured banana seedlings var. *Berangan* cv. *Intan*, confirmed that FO1, FO2, FO3, FO4, FO5 or FO6 were not pathogenic to banana seedlings. No visible foliar or internal symptoms were observed both on inoculated and control seedlings. Seedlings inoculated with FOC race 4 produced foliar symptoms as yellowing of the older leaves followed by necrosis and wilting. FO4 conferred some degree of resistance to the host when challenged with FOC race 1 suggesting the possible role of induced resistance against *Fusarium* wilt.

In this experiment, it was shown that resistance of banana seedlings var. *Berangan* cv. *Intan* against FOC race 4 was associated with inducible compounds such as peroxidases (PO) and polyphenol oxidases (PPO). There was a sharp increase in total PO and PPO activities in the early phase of infection (3-12 days) before the appearance of foliar symptoms. In the case of FOC race 4 infected activities of PO and PPO were lowest at the end of the assessment, and correspond with development of severe foliar and internal symptoms. Production of gum-like substances and formation of tyloses in xylem vessels were apparent in FO4 inoculated seedlings. Hypersensitive reactions (HR) were evident which resulted in localized infection. PO and PPO were associated with the deposition of lignin, an

important component of cell wall, chemically modified to be more resistant to cellular degrading enzymes. The proline accumulation in the leaves of FOC race 4 inoculated plants was significantly higher (at $p \geq 0.05$) when compared to those of FO4 inoculated plants and control plants throughout the duration of the experiment, suggesting, that proline could be used as a marker of stress.

Tissue-cultured

and nine days with inducer FO4 and later challenged with FOC race 4. In all treatments FO4 able to control *Fusarium* wilt disease. Disease severity (%) as marked by values of Area Under the Disease Progress Curve (AUDPC) was significantly lower compared to single inoculation with FOC race 4. An interval of nine days between inducer and challenger inoculation gave 21.8% reduction in disease severity after 54 days of challenged inoculation. Lignin accumulation in the roots was 1.2 ug/g FW as compared to 0.9 ug/g FW in non-treated seedlings based on the lignithioglycolic acid (LTGA) assay.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

KERESISTANAN TERARUH MENGGUNAKAN *FUSARIUM OXYSPORUM* BUKAN PATOGENIK UNTUK KAWALAN BIOLOGI LAYU FUSARIUM PISANG

Oleh

TARIG ELSAYED ALI ELSHARIF

February 2003

Pengerusi : **Professor Dr. Sariah Meon**

Fakulti : **Pertanian**

Pisang (*Musa* spp. Linn.) adalah tanaman buah-buahan yang penting di Malaysia. Ianya mudah di serang oleh kulat *Fusarium oxysporum* Schlect. f.sp. *cubense* (E.F.Smith) Snyder and Hansen (FOC), mengakibatkan penyakit layu vaskular di ladang. Kawalan kimia dan kultur tidak berkesan untuk mengawal penyakit ini. Oleh yang demikian kawalan alternatif perlu diusahakan. Beberapa tahun kebelakangan ini, Fusaria bukan patogenik telah dipertimbangkan untuk pegawalan penyakit tumbuhan dan mungkin merupakan pendekatan kawalan biologi terbaik untuk mengawal penyakit layu fusarium pisang melalui aruhan resistan sistemik.

F. oxysporum bukan pathogenic (FO1, FO2, FO3, FO4, FO5 dan FO6) telah di pencilkan daripada akar dan rhizosfera pokok pisang var. *Berangan* dan *Rastali* yang sakit dan dikenalpasti sehinga peringkat sepsis berdasarkan ciri kultur dan morfologi. Analisis "Random amplified polymorphic" (RAPD-PCR) membolehkan pengecaman terperinci diantara isolat *F. oxysporum* dan di antara bentuk saprofitik dan patogenik (FOC ras 1 dan FOC ras 4), menggunakan primer- primer OPC 11 dan

OPC 14. Kesemua isolat FO ini adalah antagonistik terhadap FOC (ras 1 dan ras 4) dengan peratusan perencatan pertumbuhan radial (PIRG) melebihi 50% dalam siri ujian dwikultur. FO4 telah didapati lebih antagonistik terhadap FOC race 4 dengan PIRG 65%.

Kajian kesan jangkitan ke atas anak benih kultur tisu pisang *Berangan* cv. *Intan* berumur enam minggu mengesahkan FO1, FO2, FO3, FO4, FO5 atau FO6 adalah bukan patogenik terhadap anak benih pisang. Tiada simptom pada daun atau simptom dalaman yang dapat dilihat Pada kedua-dua anak benih yang diinokulat dan kawalan. Anak benih yang dijangkiti oleh FOC ras 4 menunjukkan simptom foliar dengan daun tua menjadi kuning diikuti oleh nekrosis dan layu. FO4 dapat meningkatkan pertumbuhan vegetatif anak benih pisang dan memberi darjah keresistanan pada perumah bila dicabar dengan FOC ras 1 mencadangkan kemungkinan peranan keresistanan raruhan trhadap layu Fusarium pada anak benih yang diuji.

Dalam kajian ini menunjukkan Keresistanan anak benih pisang var. *Berangan* cv. *Intan* terhadap FOC tas 4 dikaitkan dengan kompaun perangsang, peroxidase (PO) dan polifenoloxidase (PPO). Terdapat peningkatan yang amat ketara dalam jumlah PO dan PPO di awal jangkitan (3 hingga 12 hari) sebelum kemunculan symptom foliar. Pada pokok yang dijangkiti oleh FOC ras 4, aktiviti PO dan PPO adalah sangat rendah diakhir kajian dan dikaitkan dengan pembentukan simptom foliar dan dalaman yang teruk. Penghasilan bahan seperti gam dan pembentukan tylosis dalam saluran xilem adalah jelas pada anak benih jangkitan FO4. Reaksi hipersensitif (HR), adalah jelas dan menyebabkan infeksi setempat. PO dan PPO berkait rapat dengan

pemendapan lignin, komponen penting dalan dinding sel, mengubahsuai dinding sel secara kimia menjadikan ia lebih resistan terhadap enzim pereputan selular. Pengumpulan proline dalam daun yang dijangkiti FOC ras 4 adalah lebih tinggi dan bererti (pada P≥0.05) dibandingkan dengan FO4 dan kawalan sepanjang tempoh ujikaji, mencadangkan proline boleh digunakan sebagai penanda bagi tegasan.

Anak benih pisang tisu kultur var *Berangan* cv. *Intan* yang di pra-jangkitkan selama tiga, enam dan simblan hari dengan FO4 dan kemudian dicabar dengan FOC ras 4. Dalam semua rawatan FO4 mampu mengawal penyakit layu Fusarium. Peratus keterukan penyakit yang digambarkan oleh kawasan di bawah keluk perkembangan penyakit (AUDPC) adalah rendah dibandingkan dengan anak benih yang dijangkitkan hanya dengan FOC ras 4 tempuh simblan hari di antara jangkitan perangsang dan pencabar memberikan 21.8% penurunan keterukan penyakit 54 hari selepas jangkitan cabaran. Pengumpulan lignin dalam akar ialah 1.2 ug/g berbanding dengan 0.9 ug/g dalam anak benih tanpa rawatan berdasarkan esei penentuan asid ligninthioglycolic (LTGA).

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I certify that an Examination Committee met on 5th February 2003 to conduct the final Examination of Tarig Elsyed Ali El Sharif on His Doctor of Philosophy thesis entitled "Induced Resistance using Nonpathogenic *Fusarium oxysporum* for Biological Control of Banana Fusarium Wilt" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

Zainal Abidin Mior Ahmad, Ph.D.
Lecturer,
Faculty of Agriculture,
Universiti Putra Malaysia.
(Chairman)

Sariah Meon, Ph.D.
Professor,
Faculty of Agriculture,
Universiti Putra Malaysia.
(Member)

Kamaruzaman Sijam, Ph.D.
Associate Professor,
Faculty of Agriculture,
Universiti Putra Malaysia.
(Member)

Marziah Mahmood, Ph.D.
Professor,
Faculty of Science and Environmental Studies,
Universiti Putra Malaysia.
(Member)

Baharuddin Salleh, Ph.D.
Professor,
School of Biological Sciences,
Universiti Sains Malaysia.
(Independent Examiner)

SHAMSHER MOHAMAD RAMADILI, Ph.D.
Professor/Deputy Dean,
School of Graduate Studies,
Universiti Putra Malaysia.

Date: 5 MAR 2003

This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee are as follows:

Sariah Meon, Ph.D.
Professor,
Faculty of Agriculture,
Universiti Putra Malaysia.
(Chairman)

Kamaruzaman Sijam, Ph.D.
Associate Professor,
Faculty of Agriculture,
Universiti Putra Malaysia.
(Member)

Marziah Mahmood, Ph.D.
Professor,
Faculty of Science and Environmental Studies,
Universiti Putra Malaysia.
(Member)

AINI IDERIS, Ph.D.
Professor/Dean,
School of Graduate Studies,
Universiti Putra Malaysia.

Date:

DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

TARIG ELSAYED ALI ELSHARIF

Date: 27.02.03

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LIST OF ABBREVIATIONS

µg	Microgram
ANOVA	Analysis of variance
AUDPC	Area Under Disease Progress Curve
bp	base pair
CRD	Complete Randomised Design
cfu	Colony forming units
cm	centimetre
dd	double distilled water
DMRT	Duncan's multiple range test
DNA	Deoxy ribonucleic acid
DS	Disease severity
DSI	Disease severity index
EDTA	Ethylene diaminetetra acetic acid
FA	Fusaric acid
FAO	Food and Agriculture Organization
FO	*Fusarium oxysporum*
FOC	*Fusarium oxysporum* f.sp. *cubense*
FW	fresh weight
g	Gram
ha	hectare
h	Hour
HCL	Hydrochloric acid
HR	Hypersensitive
IS	Induced resistance
ISR	Induced Systemic Resistance
Kb	Kilo-base pair
Kg	Kilogram (10^3 gram)
L	litre
LTGA	ligninthioglycolic acid
M	Molar
MARDI	Malaysia Agriculture Research and Development Institute
Min	Minute
mL	Mililiter
mm	Milimeter
mM	Milimolar
$Mmolm^{-2} s^{-1}$	milimole per meter square per second
NaCl	Sodium chloride
NaOH	Sodium hydroxide.
ng	Nanogram (10^{-9} microgram)
nm	Nanometer
NPK	Nitrogen, Phosphorous, Potassium
^{0}C	Centigrade
OPC	Oligo-nucleotide purification column primers
p	probability
PCR	Polymerase chain reaction

PIRG	percentage of inhibition of radial growth
PO	Peroxidase
PPO	polyphenol oxidase
RAPD	Random Amplified Polymorphic DNA
SAR	Systemic acquired resistance
SC	Similarity coefficient
spp.	species
TAE	Tris –HCL-glacial acetic acid-EDTA
TE	Tris-EDTA
ug	microgram (10^{-3} gram)
ul	microliter (10^{-3} ml)
UPM	Universiti Putra Malaysia
UV	Ultra violet
v/v	volume/volume
VCGs	Vegetative C complete ompatibility Groups

CHAPTER I

INTRODUCTION

Banana (*Musa* spp. (Linn.)) belonging to the Family Musaceae (Wardlaw, 1972), is the second most important fruit crop in Malaysia, accounting for 20% of the total hectarage for planted fruit (Doon, 1991; Siti Hawa, 2000). The banana industry has the potential to become one of the most profiting fruit commodities in future, marked by the increase in demand and consumption, that is expected to generate an increase in production to over 400 000 ha. by the year 2010 (Rohizad, 1998). However, land used for banana cultivation has declined over the years from 40 000 ha. in 1993 to 39 000 ha. in 2000 (Loh, 2000). Traditionally, banana cultivation is confined to smallholdings as a cash crop or as a temporary intercrop with oil palm or other crops. The popular dessert cultivars grown for domestic market are *Pisang Mas* (AA), *Pisang Embun* (AAA), *Pisang Rastali* (AAB) and *Pisang Berangan* (AAA). The cooking cultivars are *Pisang Tanduk* (AAB), *Pisang Raja* (AAB), *Pisang Nangka* (AAB), *Pisang Awak* (ABB) and *Pisang Abu* (ABB). *Pisang Mas* is the most important cultivar grown for domestic consumption and for export, followed by *Pisang Berangan*, *Pisang Embun* and *Pisang Rastali*.

Both sweet and starchy cooking types of banana called plantains, are staple food of most tropical countries. In addition, bananas and plantains provide high sources of dietary carbohydrate, vitamin C, and a number of important minerals (Tezenas, 1991). Total world production is estimated at over 76 million metric tons (FAO, 1993), of which exports (essentially of *Cavendish* bananas) to the richer nations represent less than 11 million tons. World banana production is nearly 50 million tons of which over 40% is in Asia.